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Production and Antifungal Activity of Cordytropolone and (-)-Leptosphaerone A From the Fungus *Polycephalomyces nipponicus*

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Abstract

Cordytropolone (1) and (-)-leptosphaerone A (2) were isolated from the culture broth of the fungus *Polycephalomyces nipponicus*. The structures of these two compounds were elucidated by spectroscopic methods and from a comparison of the spectroscopic data with those reported previously. The structure of 1 was confirmed by X-ray crystallography for the first time while the leptosphaerone class (compound 2) was first isolated as its (+)-antipode from the fungus *Polycephalomyces* (Cordyceps). The fermentation process was monitored weekly by High performance liquid chromatography analysis for 10 weeks. The predominant compound (1) was produced at ~0.65 mg/mg of dry extract at week 9. Compound 1 exhibited modest antipathogenic fungal activity against *Colletotrichum musae*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Fusarium* spp. TFPK301, *F. spp.* FOC1708, and *Pestalotia* spp. with percentage of mycelial growth inhibition values of $3.74 \pm 0.70\%$, $12.86 \pm 1.43\%$, $0.91 \pm 0.56\%$, $5.46 \pm 0.56\%$, $7.93 \pm 0.61\%$, and $18.75 \pm 5.24\%$, respectively, at 25 µg/mL.

Keywords

nipponicus, (-)-leptosphaerone, activity, *polycephalomyces*, production, antifungal, fungus, cordytropolone

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Abstract

Cordytropolone (**1**) and (-)-leptosphaerone A (**2**) were isolated from the culture broth of the fungus *Polycephalomyces nipponicus*. The structures of these two compounds were elucidated by spectroscopic methods and from a comparison of the spectroscopic data with those reported previously. The structure of **1** was confirmed by X-ray crystallography for the first time while the leptosphaerone class (compound **2**) was first isolated as its (+)-antipode from the fungus *Polycephalomyces* (*Cordyceps*). The fermentation process was monitored weekly by High performance liquid chromatography analysis for 10 weeks. The predominant compound (**1**) was produced at ~0.65 mg/mg of dry extract at week 9. Compound **1** exhibited modest antipathogenic fungal activity against *Colletotrichum musae*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Fusarium* spp. TFPK301, *F. spp.* FOCI708, and *Pestalotia* spp. with percentage of mycelial growth inhibition values of $3.74 \pm 0.70\%$, $12.86 \pm 1.43\%$, $0.91 \pm 0.56\%$, $5.46 \pm 0.56\%$, $7.93 \pm 0.61\%$, and $18.75 \pm 5.24\%$, respectively, at 25 $\mu\text{g/mL}$.

Keywords

Polycephalomyces nipponicus, cordytropolone, (-)-leptosphaerone A, antipathogenic fungal activity

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Cordyceps (family Cordycipitaceae) is an insect pathogenic fungus which comprises about 400 species worldwide. This genus has long been used in Chinese and Tibetan traditional medicine from ancient times. There are many reports indicating that the secondary metabolites produced by *Cordyceps* have interesting biological properties, including antioxidant,¹ anti-inflammatory,^{2,3} antimalarial,^{4–7} kidney-protective,⁸ anticancer,^{9,10} anti-bacterial,¹¹ and cytotoxic^{12,13} activities. *Cordyceps nipponica*, which has had a nomenclatural change to *Polycephalomyces nipponicus*, exhibits antibacterial¹¹ and anticancer¹⁴ activities. The isolation of cordypyrindones A–D from this species and their antimalarial and cytotoxic activities have been published.⁴ In our search for bioactive natural products from local fungi, cordytropolone (**1**) and a new natural product, (-)-leptosphaerone A (**2**), were isolated from the culture broth of *P. nipponicus*. The X-ray structure of **1** and the nuclear magnetic resonance (NMR) data of **2**, the production of **1** and **2** from this fungus, and the antipathogenic fungal activity of **1** are reported herein.

Compound **1**, a colorless amorphous powder, was obtained from the dried broth extract from a large-scale fermentation by trituration in ethyl acetate. This compound was identified as cordytropolone ($\text{C}_9\text{H}_8\text{O}_4$) (Figure 1). Its ¹H- and ¹³C-NMR spectroscopic data in DMSO-*d*₆ and MS were the same with those reported for cordytropolone from the fungus *Cordyceps* sp. BCC 1681.⁵ Crystallization of **1** from

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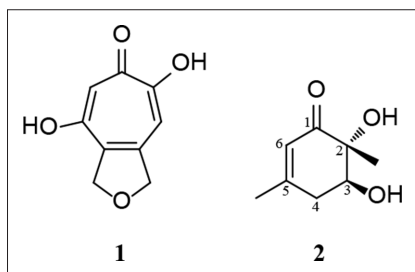


Figure 1. Structures of **1** and **2**.

methanol:water successfully provided small crystals and the structure of **1** was confirmed by X-ray crystallography for the first time (Figure 2). Considering the high amount of **1** (90% yield from the dried extract) produced by *P. nipponicus* and previous isolation (69% yield from the dried extract) from *Cordyceps* sp. BCC 1681 these two fungi are considered to be good sources of bioactive cordytropolone.

Compound **2**, a pale brownish oil, has a molecular formula of $C_8H_{12}O_3$ (m/z 179.07 $[M + Na]^+$), as determined by ESIMS. The structure of **2** (Figure 1) was elucidated on the basis of its NMR spectroscopic data in $MeOH-d_4$ (Table S1). This compound had the same structure as leptosphaerones A and B, which have been isolated from the fungus *Leptosphaeria herpotrichoides*.¹⁵ Comparison of the NMR spectroscopic data of **2** (Table S2) with those reported in the literature for leptosphaerone A were the same. However, their optical rotation values were different. Leptosphaerone A showed $[\alpha]_D + 1.9$ ($c = 0.47$, $CHCl_3$), while **2** displayed a specific rotation of the opposite sign $[\alpha]_D^{25} - 1.7$ ($c = 0.49$, $CHCl_3$). This information indicated that **2** was the enantiomer of leptosphaerone A (2*S*, 3*R*). The lack of a NOESY correlation (in $CDCl_3$, Figure S11) from the methyl protons 2- CH_3 , δ_H 1.26 (s), to the nearby oxygenated methine proton H-3, δ_H 4.00 (dd, $J = 10.5$, 6.0 Hz) supported the *trans* relationship between these protons. Hence, the stereogenic

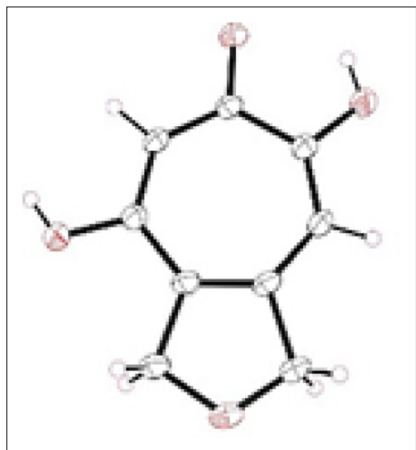


Figure 2. X-ray structure of **1**.

Table 1. Weight of Dried Mycelium and Extraction Yield of Culture Broth of the *P. nipponicus* From the Small Scale Fermentation.

Fermentation (week)	Weight of dried mycelium (g)	Extraction yield of culture broth (mg/100 mL of broth)
1	1.02	28.53
2	1.40	23.75
3	1.49	40.89
4	1.50	15.90
5	1.56	31.83
6	1.37	48.00
7	1.30	26.72
8	1.27	30.22
9	0.92	20.57
10	0.91	25.00

centers of **2** were supported as 2*R*, 3*S*. The name (–)-leptosphaerone A was given to this new compound.

The antimalarial (*P. falciparum*, K1) and cytotoxic (KB and BC-1 cell lines) activities of cordytropolone,⁵ and the cytotoxicity (A-549 cell lines) of leptosphaerone C (same identified structure with leptosphaerone A) isolated from the fungus *Penicillium* sp. have already been published.¹⁶

A time course of a small-scale fermentation of *P. nipponicus* for 10 weeks was conducted in order to study the production of **1** from the culture broth of this fungus. From our experiment, the weight of the dried mycelium varied from 0.91 to 1.56 g. It obviously increased from weeks 1 to 3, slightly went up from weeks 3 to 5, and then started to decrease from weeks 5 to 10 (Table 1).

The production of **1** and **2** in the broth extracts was monitored by high performance liquid chromatography (HPLC) analysis using pure compounds **1** (t_R 2.52 min) and **2** (t_R 11.20 min), and also adenine (t_R 7.63 min) and adenosine (t_R 12.35 min) as reference standards (Figures S13–S16). This study showed that compounds **1** and **2** were produced by *P. nipponicus* in every week of the fermentation, as well as adenine and adenosine (Table 2). The quantity of **1** in the extracts was determined from its peak area calculated based on a standard linear equation of pure compound **1** (r^2 0.988), which showed the highest at week 9 (~0.65 mg/mg of dried extract). Surprisingly, the production of **1** at week 3 (21 days), the optimal fermentation condition for growing this fungus on induced medium, was very low when compared with most other weeks. These results were different from our large-scale fermentation (20 days) results. Therefore, a second large-scale fermentation was started and the isolation of **1** under the same procedure was repeated. Only 756.2 mg (55% yield from 1.38 g of the dried extract) of **1** was obtained. These results confirmed that **1** is

Table 2. Production of **1**, **2**, Adenine and Adenosine From Small-Scale Fermentation of *P. nipponicus*.

Fermentation (week)	Production of compound			
	1 (mg/mg of dried extract)	2	Adenine	Adenosine
1	0.08	+	+	+
2	0.12	+	+	+
3	0.07	+	+	+
4	0.03	+	+	+
5	0.35	+	+	+
6	0.22	+	+	+
7	0.10	+	+	+
8	0.48	+	+	+
9	0.65	+	+	+
10	0.31	+	+	+

+ indicates detectable in the broth extracts.

produced by this fungus, but the compound is not stable under the same fermentation conditions.

The antifungal activity of **1** at 25 µg/mL was tested against 6 plant pathogenic fungi. The results revealed that **1** had a slight inhibitory effect against fungal mycelial growth, as shown in Table 3. The antifungal activity of **1** against plant pathogenic fungi including *Colletotrichum capsici* and *Colletotrichum gloeosporioides* has been reported recently.¹⁷ However, the mechanisms of action against fungal pathogens have not yet been described.

Compound **2** was tested for its cytotoxicity against human breast cancer (MCF-7), oral human epidermoid carcinoma cancer (KB) and Vero (African green monkey kidney fibroblasts) cell lines, and antiviral activity against Herpes simplex virus type-1 (HSV-1) at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. It was found to be inactive in all these tests.

Cordytropolone (**1**) and the new natural product (–)-leptosphaerone A (**2**) were isolated from the culture broth of the fungus *P. nipponicus*. The structure of **1** was confirmed by X-ray crystallography for the first time. The NMR spectroscopic data of **2** and the isolation of the leptosphaerones from the fungus *Polycephalomyces (Cordyceps)* are reported

for the first time. Compounds **1** and **2** were produced regularly during 10 weeks of fermentation, but the content of **1** was not stable. The antifungal activity of **1** was considered weak against 6 plant pathogenic fungi, while **2** was found to lack cytotoxicity against MCF-7 and KB cancer cell lines and was also inactive against HSV-1. Owing to the high quantity of **1** produced by this fungus, fermentation conditions that can lead to the stable production of **1** and structure modification of this compound on its biological activity are our interests.

Experimental

General

¹H-NMR (500 MHz), ¹³C-NMR (125 MHz), and 2D NMR spectra were recorded in DMSO-*d*₆ for **1**, and MeOH-*d*₄ and CDCl₃ for **2** on a Bruker 500-MHz Avance Neo NMR Spectrometer. Optical rotations were determined using a JASCO DIP-1000 digital polarimeter. Mass spectra were obtained from a Bruker micrOTOF mass spectrometer. Column chromatography was carried out on Merck silica gel 60 and Pharmacia Fine chemicals Sephadex G-75. HPLC was performed on a Shimadzu system with a SLC-10AD controller and detector using a diode array detector (SPD-M20A; Shimadzu). A C18 column (250 × 4.6 mm, 5 µm) was used in this analysis.

Fungal Material and Fermentation

Polycephalomyces nipponicus (Cod-MK1201) was isolated from a dead cicada nymph and collected from Muang District, Maha Sarakham Province, northeast Thailand. The fungus was isolated and identified by one of the authors (A.S.). The culture used throughout the experiment was maintained on potato dextrose agar (PDA) slants at 28°C. For inoculum preparation, the fungus was initially grown at 28°C on a PDA plate for 14 days. The hyphal tip of the fungal strain was cut with a sterile cork borer and transferred to 25 mL of induced medium (35 g/L of sucrose, 5 g/L of peptone, 2.5 g/L of yeast extract, 0.5 g/L of MgSO₄, 1 g/L of KH₂PO₄, and 0.05 g/L of vitamin B1, adjusted to pH 5.2 in a 250 mL flask, and grown at 28°C.¹⁸ The culture broth was

Table 3. the Percentage of PGI of **1** Against Six Plant Pathogenic Fungi.

Fungal strains	Original host plant	The percentage (%) of mycelial growth inhibition
<i>Colletotrichum musae</i>	Cultivated banana	3.74 ± 0.70
<i>Colletotrichum capsici</i>	Papaya	12.86 ± 1.43
<i>Colletotrichum gloeosporioides</i>	Mango	0.91 ± 0.56
<i>Fusarium</i> spp. TFPK301	Tomato	5.46 ± 0.56
<i>Fusarium</i> spp. Foc 1708	Banana	7.93 ± 0.61
<i>Pestalotia</i> spp.	Mango	18.75 ± 5.24

collected from 5 flasks every week for the small-scale time course fermentation (1, 10 weeks), while for the large-scale fermentation, 100 flasks were collected at day 20. The culture broth was filtered through a 0.2 μm filter membrane before extraction.

Extraction and Isolation

For the small-scale extraction, the culture broth was extracted twice with an equal volume of ethyl acetate. The combined ethyl acetate layer was concentrated under reduced pressure to obtain an extract which was further subjected to analyses by HPLC. For the large-scale extraction, the culture broth (2.5 L) was divided into 5 portions (500 mL) and each portion was extracted with ethyl acetate (300 mL, $\times 3$). The collected ethyl acetate layer was combined and concentrated under reduced pressure to obtain the ethyl acetate extract (8.9 g). This was further triturated in ethyl acetate at room temperature and stirred for 24 hours (50 mL, $\times 2$) to yield **1** (8.0 g) and an ethyl acetate-soluble fraction (520 mg). The ethyl acetate-soluble fraction was subjected to purification by silica gel and Sephadex G-75 CC to give **2** (12.3 mg) as a pale brownish oil.

(-)-Leptosphaerone A (**2**)

Pale brownish oil.

$[\alpha]_D^{25}$: -1.7 ($c = 0.49$, CHCl_3).

^1H NMR (500 MHz, $\text{CH}_3\text{OH}-d_4$): 5.88 (1H, s, H-6), 3.91 (1H, dd, $J = 9.5, 5.5$ Hz, H-3), 2.67 (1H, dd, $J = 18.5, 5.5$ Hz, H-4eq.), 2.41 (1H, dd, $J = 18.5, 5.5$ Hz, H-4ax.), 2.00 (3H, s, 5- CH_3), 1.22 (3H, s, 2- CH_3).

^{13}C NMR (125 MHz, $\text{CH}_3\text{OH}-d_4$): 203.7 (C, C-1), 162.5 (C, C-5), 125.0 (CH, C-6), 78.3 (C, C-2), 73.9 (CH-C-3), 39.4 (CH_2 , C-4), 24.4 (CH_3 , 5- CH_3), 18.1 (CH_3 , 2- CH_3).

ESIMS: m/z 179.07 [$M + \text{Na}$] $^+$ ($\text{C}_8\text{H}_{12}\text{O}_3\text{Na}$)

HPLC Analysis

The extract samples for HPLC were prepared at 2 mg/mL in methanol: water (1:1) while the standard compounds were prepared at 100 $\mu\text{g/mL}$ in water. Each sample solution of 50 μL was injected onto a reversed phase C18 column. Methanol: milli-Q water (15:85 v/v) was used as a mobile phase with a constant flow rate of 1 mL/min. The column temperature was set at 30°C and the total run time was 30 min. All samples were detected at 254 nm using a diode array detector.

Antifungal Activity Assay (Pore Plate Technique)

Compound **1** (5 mL) as a solution in water at a concentration of 500 $\mu\text{g/mL}$ was mixed in 95 mL PDA medium before being plated onto 90 mm Petri dishes. Seven-day-old mycelial discs of 6 plant pathogenic fungal pathogens

(*Colletotrichum musae*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Pestalotia* spp., *Fusarium* spp. TFPK301 and *Fusarium* spp. Foc 1708) were cut with a 7 mm sterilized cork borer under aseptic conditions and placed onto 25 mL PDA plates containing 25 $\mu\text{g/mL}$ (final concentration) of **1**. The plates were incubated at 28°C and the mycelial growth was determined at day 7. The percentage of mycelial growth inhibition (PGI) was calculated using the formula; $\text{PGI} (\%) = R - R_1/R \times 100$, where R represents the fungal growth radius (mm) of the control culture and R_1 represents the fungal growth radius distance (mm) in the treatment culture.¹⁹ The experiment was replicated 5 times, and the fungus grown on the PDA plate was used as the control.

Biological Assay

Anti- HSV-1 and cytotoxicity assays against human breast cancer (MCF-7), oral human epidermoid carcinoma (KB), and Vero (African green monkey kidney fibroblasts) cell lines were evaluated using a colorimetric method.²⁰

Crystal Data of **1**

A single crystal of **1** was mounted on the end of a hollow glass fiber. X-ray diffraction data were collected using a Bruker D8 VENTURE and operating at $T = 296(2)$ K. Data were measured using ω and ϕ scans and using Cu-K α radiation ($\lambda = 1.54056$ Å). The total number of runs and images was based on the strategy calculation from the program APEX3 and unit cell indexing was refined using SAINT (V8.38A, Bruker, 2016). Data reduction and scaling were performed using SAINT (V8.38A), and SADABS-2016/2 was used for absorption correction (APEX3, SADABS and SAINT; Bruker AXS Inc., Madison, Wisconsin, USA, 2016). The structure was solved with the ShelXT structure solution program using combined Patterson and dual-space recycling methods.²¹ The structure was refined by least squares using ShelXL.²² All non-hydrogen atoms were refined anisotropically. The hydrogen atoms of organic ligands were placed in calculated positions and refined using a riding model on attached atoms with isotropic thermal parameters 1.2 times those of their carrier atoms. The O-H hydrogen atoms were located in difference Fourier maps, but refined with O-H = 0.82 ± 0.01 Å. The data have been deposited with the Cambridge Crystallographic Data Center (CCDC) with CCDC number 1 843 739.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

1. SP L, Li P, Dong TTX, Tsim KWK. Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. *Phytomedicine*. 2001;8(3):207-212.
2. Kim HG, Shrestha B, Lim SY, et al. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF- κ B through Akt and p38 inhibition in RAW 264.7 macrophage cells. *Eur J Pharmacol*. 2006;545(2-3):192-199.
3. Chiu CP, Liu SC, Tang CH, et al. Anti-inflammatory cerebroside from cultivated *Cordyceps militaris*. *J Agric Food Chem*. 2016;64(7):1540-1548.
4. Isaka M, Tanticharoen M, Kongsaree P, Thebtaranonth Y. Structures of cordypyridones A-D, antimalarial *N*-hydroxy- and *N*-methoxy-2-pyridones from the insect pathogenic fungus *Cordyceps nipponica*. *J Org Chem*. 2001;66(14):4803-4808.
5. Seephonkai P, Isaka M, Kittakoop P, et al. A new tropolone from the insect pathogenic fungus *Cordyceps* sp. BCC 1681. *J Antibiot*. 2001;54(9):751-752.
6. Rukachaisirikul V, Pramjit S, Pakawatchai C, Isaka M, Supothina S. 10-membered macrolides from the insect pathogenic fungus *Cordyceps militaris* BCC 2816. *J Nat Prod*. 2004;67(11):1953-1955.
7. Isaka M, Boonkhao B, Rachtawee P, Auncharoen P. A xanthocillin-like alkaloid from the insect pathogenic fungus *Cordyceps brunnearubra* BCC 1395. *J Nat Prod*. 2007;70(4):656-658.
8. Wang Y, Yin H, Lv X, Wang Y, Gao H, Wang M. Protection of chronic renal failure by a polysaccharide from *Cordyceps sinensis*. *Fitoterapia*. 2010;81(5):397-402.
9. Aramwit P, Bang N, Ratanavaraporn J, Nakpheng T, Srichana T. An anti-cancer cordycepin produced by *Cordyceps militaris* growing on the dead larva of *Bombyx mori* silkworm. *J Agric Sci*. 2014;6(6):41-53.
10. Nakamura K, Shinozuka K, Yoshikawa N. Anticancer and antimetastatic effects of cordycepin, an active component of *Cordyceps sinensis*. *J Pharmacol Sci*. 2015;127(127):53-56.
11. Sangdee K, Nakbanpote W, Sangdee A. Isolation of the entomopathogenic fungal strain Cod-MK1201 from a cicada nymph and assessment of its antibacterial activities. *Int J Med Mushrooms*. 2015;17(1):51-63.
12. Bunyapaiboonsri T, Yoiprommarat S, Intereya K, Kocharin K. New diphenyl ethers from the insect pathogenic fungus *Cordyceps* sp. BCC 1861. *Chem PharmBull*. 2007;55(2):304-307.
13. Wang J-hui, Zhang Z-liang, Wang Y-qin, et al. Chemical constituents from mycelia and spores of fungus *Cordyceps cicadae*. *Chinese Herbal Medicines*. 2017;9(2):188-192.
14. Sangdee K, Seephonkai P, Buranrat B, Surapong N, Sangdee A. Effects of ethyl acetate extracts from the *Polycephalomycetes nipponicus* isolate Cod-MK1201 (Ascomycetes) against human pathogenic bacteria and a breast cancer cell line. *Int J Med Mushrooms*. 2016;18(8):733-743.
15. Ayer WA, Gökdemir T, Miao S, Trifonov LS, Leptosphaerones A. Leptosphaerones A and B, new cyclohexenones from *Leptosphaeria herpotrichoides*. *J Nat Prod*. 1993;56(9):1647-1650.
16. Lin Z, Zhu T, Fang Y, Gu Q, Zhu W. Polyketides from *Penicillium* sp. JP-1, an endophytic fungus associated with the mangrove plant *Aegiceras corniculatum*. *Phytochemistry*. 2008;69(5):1273-1278.
17. Jaihan P, Sangdee K, Sangdee A. Disease suppressive activity of extracts from entomopathogenic fungus *Ophiocordyceps sobolifera* against chili anthracnose fungi *Colletotrichum* spp. in a pot experiment. *J Gen Plant Pathol*. 2018;84(3):237-242.
18. Huang L, QZ L, Chen YY, Wang XF, Zhou XW. Determination and analysis of cordycepin and adenosine in the products of *Cordyceps* spp. *Afr J Microbiol Res*. 2009;3:957-961.
19. Kumer AS, Eswara Reddy NP, Hariprasad Reddy K, Charitha Devi M. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in agri export zone of Andhra Pradesh, India. *Plant Pathol Bull*. 2007;16:157-160.
20. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst*. 1990;82(13):1107-1112.
21. Sheldrick GM. SHELXT - integrated space-group and crystal-structure determination. *Acta Crystallogr A Found Adv*. 2015;71(1):3-8.
22. Sheldrick GM. Crystal structure refinement with SHELXL. *Acta Crystallogr C Struct Chem*. 2015;7(Pt 1):3-8.